wild pairs" represents a minimum, for it appears likely that pairs with only one of each necessary gene between them would produce far fewer than 1 crossveinless fly in 1000 F_2 flies.

Support for two suggestions is lent by this experiment: (i) that of Dobzhansky and his co-workers that an individual often possesses an unexpectedly great portion of the total genetic variation of its population (*5*); (ii), that the presently observable steps in evolution are made through new combinations of common genes (*5*), which have therefore already been long since tried and tested by natural selection as members of an individual's team of genes (*6*).

Finally, it is suggested that results such as these should be borne in mind in the consideration of the many polygenic traits in human beings.

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Relationship of Stress-Induced Histidine Decarboxylase to Circulatory Homeostasis and Shock

Abstract. Histidine decarboxylase activity of mouse tissues is increased by stress and by injection of epinephrine and norepinephrine, suggesting a balance between histamine and catechol amines producing a component of circulatory homeostasis. Imbalance during intense stress might lead to failure of circulatory homeostasis and to shock. Reasons for discounting histamine as "shock toxin" may be invalid.

Workers in this laboratory have recently demonstrated that mammalian histidine decarboxylase is an adaptive enzyme (*1*); its activity in animal tissues increases in response to nonspecific stress, for example, treatment with histamine liberators, burns, delayed allergy, treatment with pertussis vaccine (*2*), exposure to cold, and injection with *Escherichia coli* endotoxin (*3*).

Among the most firmly established consequences of stress are discharge of epinephrine from the adrenal and release of norepinephrine from the sympathetic nerve endings. The catechol amines seemed likely chemical mediators of stress-induced histidine decarboxylase activity and were consequently tested.

Mice were injected intramuscularly with 20 μg of epinephrine in oil; controls received oil only. The animals were killed after 6 hours, and tissues were assayed for histidine decarboxylase activity (*4*). Results for skin of control mice were 87, 96, and 117 (av., 100); for skin of epinephrine-treated mice, 339, 348, and 466 (av., 384). Results for lungs of control mice were 42, 94, and 163 (av., 100); for lungs of epinephrine-treated mice, 367, 408, and 510 (av., 428). Thus the histidine decarboxylase activity of mouse skin and lung was increased fourfold (*5*). This has been repeatedly confirmed. Histidine decarboxylase activity of muscle was increased threefold under these conditions; other tissues are under investigation.

Results of a time study of the effects of 20 μg epinephrine in oil were as follows: for skin of control mice, 96, 97, and 107 (av., 100); for mice killed 1 hour after injection, 74, 75, and 84 (av., 78); for mice killed 6 hours after injection, 447, 454, and 575 (av., 492); and for mice killed 24 hours after injection, 97, 104, and 139 (av., 113).

Norepinephrine also increased histidine decarboxylase activity. Mice were injected subcutaneously with aqueous solutions of DL-norepinephrine equivalent to either 20 μg or 60 μg of the *l*-isomer. Each received three injections 2 hours apart. After 6½ hours the skins were assayed. For controls the values were 89, 104, and 107 (av., 100); for mice receiving three 20-μg doses of norepinephrine, 253, 312, and 375 (av., 313); for mice receiving three 60-μg doses of norepinephrine, 436, 493, and 593 (av., 507).

The earlier demonstration (*2*) that activity of histidine decarboxylase increases in response to diversified forms of stress becomes more comprehensible in view of the fact that catechol amines, known to be released in stress, also increase enzyme activity. These observations suggest that there may be a balance between the two catechol amines on the one hand, and newly synthesized histamine on the other, producing a component of circulatory homeostasis which operates under conditions of stress.

It is further suggested that in those conditions of stress where there is ultimately a failure in circulatory homeostasis, for example, traumatic shock, one of these amines may be a causative factor.

The intense adrenergic stimulation preceding stress-induced shock is well recognized; now we have demonstrated that stress increases the activity of the enzyme which synthesizes histamine. Although it has not been proved that increased in vitro histidine decarboxylase activity in the mouse is a measure of the rate of histamine synthesis in the tissues of the living animal, (*6*, *7*) this has been done in the rat (*1*). The importance of the shock problem and the attractiveness of a concept involving new formation of histamine seem to justify speculation based on the assumption that stress increases histamine synthesis in vivo. The hypothesis is therefore proposed that if during stress the supply of either histamine or the catechol amines fails, the remaining amine may be extraordinarily toxic to the cells of the small blood vessels and cause shock. The lethal effect of either amine when acting on such cells in the absence of its natural antagonist may be much greater than heretofore suspected, since in experimental tests on these drugs there is probably always some compensation by release or formation of the antagonist.

There are two obvious possibilities for explaining certain features of some types of shock in terms of an imbalance in the catechol amine-histamine relationship. First, histamine may not be formed in adequate supply in strategic locations, so that it cannot cope with catechol amines from the adrenals and sympathetic nerve endings. In this case, excessive vasoconstriction may produce hypoxia with resulting damage to the cells of the small blood vessels.

Second, intense stress may result ultimately in some degree of depletion of the catechol amines from their depots. Thus a highly active mechanism for synthesizing histamine might be left in operation. Injected histamine can produce shock in animals of many species; if formed throughout major tissues of an animal whose defenses are to some extent exhausted, it may cause severe damage.

Adrenal steroids released during stress also oppose some actions of histamine in some species; however, the degree of depletion and rate of resynthesis of these steroids during stress is not clear and their role cannot be evaluated at this time.

In certain types of shock there is good reason to believe that histamine may be the shock "toxin." Adrenalectomized animals are highly sensitive to shock and to histamine; yet they

can be injected with large amounts of epinephrine and norepinephrine. Cortisone, which antagonizes some effects of histamine, is reported to protect adrenalectomized rats from traumatic shock (*8*). In many cases of shock, adrenergic stimulation is probably extremely strong in the early stages before histidine decarboxylase has reached full activity; yet often the death of the animal is delayed. During long-continued infusions of epinephrine in man and some other species a strong drop in blood pressure occurs; if the infusion is abruptly terminated, shock may ensue (*9*). Finally, bacterial endotoxins produce shock closely resembling stress shock (*10*); *Escherichia coli* endoxin is an extremely strong inducer of histidine decarboxylase activity in many tissues (*3*).

Other possibilities relating the histamine-catechol amine balance to shock are that different tissues of the same animal may be injured by different amines, or, that once a cell is injured, all the amines may be toxic to it.

In the early research on shock, numerous workers considered histamine, or histamine-like substances, as likely candidates to be shock "toxin" (*11*). It has been dropped almost entirely from consideration at the present time. Some of the reasons are the following: (i) there are differences in the characteristics of stress-induced shock and shock produced by injection of histamine; (ii) the histamine content of normal muscle is too low to cause shock when the muscle is traumatized; (iii) histamine is not consistently found in blood and lymph in increased quantities during shock; (iv) antihistamines do not protect against shock.

Recent findings suggest that histamine may be newly synthesized at an increasing rate during stress, that it may act for long periods of time, that it may be formed close to, or possibly even within the cells which it stimulates, and that it is rapidly inactivated, forming metabolites for which there are no suitable analytical procedures (*7*). If these conjectures are correct, the reasons for discounting histamine as a shock "toxin" can no longer be considered valid (*12*).

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4. Histidine decarboxylase was assayed by incubation of tissue extracts with 10 μg of C^{14} L-histidine for 3 hours at 37°C and determining the C^{14} histamine formed by addition of carrier, isolation, conversion to dibenzenesulfonylhistamine, and counting in a liquid scintillation counter. The method has been described in detail (*1*). All values for enzyme activity were recalculated so that the average of the controls is arbitrarily 100. This permits easier comparison of results of experiments done at different times and under different conditions.
5. There are at least two distinct types of histidine decarboxylase according to function; one is in mast cells and certain other unidentified cells and produces histamine which is largely bound. The second is the inducible histidine decarboxylase; its anatomical location is not known. The histidine decarboxylase in tissues of normal animals may be largely, perhaps entirely, due to the first type; hence the increase in the inducible histidine decarboxylase activity may be much greater than indicated by the data.
6. Histamine catabolism is complex in mice (*11*) and there is no satisfactory method for measuring histidine decarboxylase activity in vivo in this species. Therefore, only in vitro studies were made. In female rats histamine catabolism is simpler, and in these animals we have previously shown that results of in vitro experiments on induced histidine decarboxylase are parallel to those obtained from experiments in living animals (*1*).
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Critical Periods for the Effects of Infantile Experience on Adult Learning

Abstract. Mice were shocked with 0.1, 0.3, or 0.5 ma of current at 2 to 3, 8 to 9, or 15 to 16 days. Handled, nonshocked and nonhandled controls were also used. In adulthood each group was split into thirds and taught an avoidance response under shock of 0.3, 0.5, or 0.7 ma. The amount of shock given during infancy and adulthood, and the age at which shock occurred, were all found to have significant effects upon learning.

Several investigators have recently studied the question of critical periods in infancy (*1*). On the behavioral level, Schaefer (*2*) has reported that rats handled during the first week of life exhibit less emotionality in adulthood than animals handled at other times. Denenberg has shown that rats handled during the first 10 days of life are better avoidance learners in adulthood than rats handled during the second 10 days or the first 20 days of life (*3*), and that mice shocked at different times during early life have differential adult conditioning scores as well as different response topologies (*4*). On the physiological level, Levine and Lewis (*5*) have determined that rats manipulated (handled) at ages 2 to 5 days and 2 to 13 days exhibit significant adrenal ascorbic-acid depletion when assayed at 14 days, but that animals handled at 6 to 9 or 10 to 13 days do not show any evidence of depletion. Denenberg and Karas (*6*) used rats and mice which were either not handled at all or were handled for the first 10, the second 10, or the first 20 days of life; the groups handled for 20 days weighed the most, but animals handled for the first 10 days lived longest under conditions of total food and water deprivation.

It has also been shown that shock administered to mice at 25 days will significantly affect 50-day conditioning scores (*7*), and that shock administered on two days between the 5th and 10th days of life will lead to more rapid extinction of a learned response (*4*). However, there has been no systematic study of the relatively long-term behavioral effects of stimulation given to restricted age groups at different critical periods in infancy. We wish to describe some of the findings of such a study (*8*).

The subjects were 290 mice (strain C57BL/10Sc). They were stimulated at one of three ages: 2 to 3 days, 8 to 9 days, or 15 to 16 days. These ages are at the mid-point of the first three critical periods specified by Williams and Scott (*9*) and specified with modifications by one of us (*4*). Stimulation consisted of removing the complete litter from the home cage, placing the pups on a grid, and subjecting them to one of three levels of constant current: 0.1, 0.3, or 0.5 ma. Ten 1-second shocks were administered, with a 45-second pause between shocks. Handled, nonshocked controls (0.0 ma) were treated in the same way as shocked mice, except for lack of current on the grid. In addition, other litters served as nonhandled, nonshocked controls. All litters were weaned at 22 days and reared thereafter in small cages with littermates of like sex. At 61 days of age the 13 groups were randomly split into thirds and received avoidance learning conditioning under shock of 0.3, 0.5, or 0.7 ma. They received six trials a day for 7 days. The conditioning consisted of the sounding of a buzzer, followed 5 seconds later by shock. If the mouse made the appropriate response prior to the onset of shock, the shock did not occur and the mouse was credited with an "avoidance response."

Figure 1 presents the mean number of avoidance responses as a function of the level of shock given during adulthood for each of the three critical periods. Separate graphs are given for each level of shock given during infancy. The curve for the nonhandled, nonshocked control groups is based on the same mice in each of the graphs. With this one exception, each point of